UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

August 20, 2011

MEMORANDUM

Subject: Efficacy Review for Opti-Cide 3; EPA Reg. No. 70144-1; DP Barcode: D390328

From: Ibrahim Laniyan, Ph.D.
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Regulatory Management Branch I
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Applicant: Micro-Scientific Industries, Inc.
1225 Carnegie Street
Rolling Meadows, IL 60008

Formulation from the Label:

Active Ingredients	% by wt.
n-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride	0.154 %
n-Alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chloride	0.154 %
Other Ingredients:	99.692 %
Total	100.000 %

I. BACKGROUND

The product, Opti-Cide 3 (EPA Reg. No. 70144-1), is an EPA-approved disinfectant (bactericide, fungicide, tuberculocide, virucide) for use on hard, non-porous surfaces in household, commercial, institutional, food processing, food service, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add new claims for effectiveness as a sanitizer on hard, non-porous surfaces and soft surfaces; and to add 2-minute "kill" claims for selected microorganisms. The product is for use on pre-cleaned surfaces. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and MICROBIOTEST, located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to EPA (dated May 12, 2011), EPA Form 8570-35 (Data Matrix), eleven studies (MRID 484820-01 through 484820-11), Statements of No Data Confidentiality Claims for all eleven studies, a copy of the last accepted label (dated December 2, 2010), and the proposed label.

II. USE DIRECTIONS

The product is designed for disinfecting and sanitizing surfaces. The product may be used to treat hard, non-porous surfaces, including: air vents, aquariums, armrests, ATM buttons, bar tops, bath tubs, bedrails, benches, biohazard team equipment, biological monitoring devices, bowls, bridles, brushes, cabinets, cages, call buttons, carts, cash registers, chainsaw blades, changing tables, clipper blades, clippers, coin dispensers, combs, computer keyboards, computer screens, cots, counters, CPR training devices, CPR training manikins, curing lights, cuspidors, cutting boards, decks, dental equipment (as specified on the proposed label), diaper changing tables, diaper pails, dictating machines, diving equipment and masks, door handles, doorknobs, drinking fountains, dry suits, dumbbells, elevator buttons, elevator panels, empty whirlpool tanks, escalator handrails, face shields, faucets, fish tank equipment (as specified on the proposed label), fixtures, floors, flower pots, foot spa bowls, furniture (as specified on the proposed label), gaming machines, garbage cans, garbage compactors, garden equipment, goggles, grass mower blades, grooming tables, gym mats, handcuffs, handrails, harnesses, hospital and medical equipment (as identified on the proposed label), kennels, leashes, light lens covers, light pull switches, lights, litter boxes, lockers, manicure implements, masks, mats, microscopes, monitors, optical wear (excluding contact lenses), pedicure equipment, play-care equipment, razors, remote controls, reptile habitats, scales, scissors, seats, shower doors, showers, sinks, slot machines, soap containers, soap dishes, stands, stools, tables, tanning beds, telephones, toilets, tools, toys, trash cans, tree saws, walls, weight machines, wet suits, and window sills. The product may be used to treat soft surfaces, including: cloth furniture, coated mattresses, coated pillows, curtains, leather surfaces, mattress covers, and upholstered fixtures. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: coated surfaces, glass, laminated surfaces, metal (e.g., aluminum, brass, stainless steel), painted surfaces, plastic (e.g., acrylic, polycarbonate, polypropylene, polystyrene, polyvinylchloride, vinyl), and Plexiglas. Directions on the proposed label provide the following information regarding use of the product:

> As a disinfectant: Apply the product to pre-cleaned surfaces. Allow to remain wet for 2, 3, or 5 minutes (as appropriate). Wipe dry using a clean paper or cloth towel; or rinse surfaces using potable water and either wipe surfaces dry or allow to air dry.

As a sanitizer: Apply the product to pre-cleaned surfaces. Allow to remain wet for 10 second. Wipe dry using a clean paper or cloth towel; or rinse surfaces using potable water and either wipe surfaces dry or allow to air dry.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use in Hospital or Medical Environments; Batch Replication for Modified Test; Different Exposure Period: Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support supplemental efficacy claims for fungicides, tuberculocides, and virucides under modified conditions (e.g., different exposure period). Additional testing may be conducted with reduced batch replications. Specifically, data may be developed on the applicant's own finished product, at the same use concentration, for one product sample, instead of two product samples.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method): Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain

chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizers (For Non-Food Contact Surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 484820-01 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modified for Spray Product Application) Against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048)" for Opti-Cide 3, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – July 14, 2010. Laboratory Project Identification Number A09643.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. 21090, 21330, and 30200) of the product, Opti-Cide 3, were tested using ATS Laboratory Protocol No. MSI01052710.NFS (copy provided). At least one of the product lots tested (i.e., Lot No. 21330) was at least 60 days old at

the time of testing. The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) per product lot per microorganism were inoculated with 10.0 µL of a 51-53 hour old suspension of test organism. The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 20-30 minutes at 35-37°C at 40% humidity. Each carrier was transferred to a plastic Petri dish and was sprayed (2 pumps) with the product from a distance of 4-6 inches from the carrier surface until drenched. The carriers were allowed to remain wet for 10 seconds at 21°C. Following exposure, each carrier was placed into a sterile vessel containing 20 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. Following neutralization, the excess liquid from each plastic Petri dish was transferred to the corresponding neutralizer vessel containing the corresponding carrier. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. *Staphylococcus aureus* cultures were incubated for ~44 hours at 35-37°C. *Enterobacter aerogenes* cultures were incubated for ~44 hours at 25-30°C. All subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed.

2. MRID 484820-02 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Surface Non-Food Contact Surfaces (Modified for Spray Product Application) Against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048)" for Opti-Cide 3, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – September 15, 2010. Laboratory Project Identification Number A10045.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Two lots (Lot Nos. 21330, and 31620) of the product, Opti-Cide 3, were tested using ATS Laboratory Protocol No. MSI01072110.NFS (copy provided). At least one of the product lots tested (i.e., Lot No. 21330) was at least 60 days old at the time of testing. The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Five sterile fabric carriers (1 inch x 1 inch) per product lot per microorganism were inoculated with 10.0-20.0 µL of a 48±4 hour old suspension of test organism. The carriers were dried for 20 minutes at 35-37°C at 42% humidity. Each carrier was transferred to a plastic Petri dish and was sprayed (4 pumps) with the product from a distance of 4-6 inches from the carrier surface until drenched. The carriers were allowed to remain wet for 10 seconds at 21°C. Following exposure, each carrier was placed into a sterile vessel containing 20 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. Following neutralization, the excess liquid from each plastic Petri dish was transferred to the corresponding neutralizer vessel containing the corresponding carrier. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. *Staphylococcus aureus* cultures were incubated for ~44 hours at 35-37°C. *Enterobacter aerogenes* cultures were incubated for ~44 hours at 25-30°C. All subcultures were stored for 1 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Protocol amendment reported in the study was reviewed.

3. MRID 484820-03 "AOAC Germicidal Spray Test - Healthcare Against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442) and *Salmonella enterica* (ATCC 10708)" for Opti-Cide 3, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Study completion date – August 31, 2010. Laboratory Project Identification Number 485-145.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 21090, 22010, and 22659N) of the product, Opti-Cide 3 Disinfectant, were tested using MicroBiotest Protocol No. 485.1.07.27.10 (copy provided). At least one of the product lots tested (i.e., Lot No. 22659N) was at least 60 days old at the time of testing. The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Sixty (60) glass slide carriers (1 inch x 3 inches) per product lot per microorganism were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (3 sprays) from a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 2 minutes at 19°C. Following the exposure period, the remaining liquid was drained from each carrier. Following the exposure period, individual carriers were transferred to tubes containing DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for 48±2 hours at 37±2°C. Due to the opacity of the neutralizer, all *Pseudomonas aeruginosa* subcultures were streaked onto tryptic soy agar and incubated for 24±2 hours at 37±2°C. Following incubation, the subcultures and streaks were examined for the presence or absence of visible growth. Controls included those for carrier counts, confirmation of the challenge microorganisms, sterility, viability, neutralizer effectiveness, and bacteriostasis.

Note: Protocol deviations/amendments reported in the study were reviewed.

4. MRID 484820-04 "AOAC Tuberculocidal Activity of a Germicidal Spray - Initial Against *Mycobacterium bovis*, BCG" for Opti-Cide 3, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – November 30, 2010. Laboratory Project Identification Number 485-146.

This study, under the direction of Study Director M. Hamid Bashir, was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation). Two lots (Lot Nos. 21330 and 22010) of the product, Opti-Cide 3 Disinfectant, were tested using MicroBiotest Protocol No. 485.2.07.27.10 (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inches) per product lot were inoculated with 0.02 mL of a 21-25 day old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product from a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 2 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. The carriers were transferred to individual tubes containing 20 mL of DE Neutralizing Broth. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes

containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 60 days at 37±2°C. The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, confirmation of the challenge microorganism, sterility, viability, and neutralizer effectiveness.

Note: Protocol deviations/amendments reported in the study were reviewed.

5. MRID 484820-05 "AOAC Tuberculocidal Activity of a Germicidal Spray - Confirmatory Against *Mycobacterium bovis*, BCG" for Opti-Cide 3, by Angela L. Holingsworth. Study conducted at MICROBIOTEST. Study completion date – November 30, 2010. Laboratory Project Identification Number 485-147.

This confirmatory study, under the direction of Study Director Angela L. Holingsworth, was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation). Two lots (Lot Nos. 21330 and 22010) of the product, Opti-Cide 3 Disinfectant, were tested using MicroBiotest Protocol No. 485.3.07.27.10 (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) glass slide carriers (1 inch x 3 inches) per product lot were inoculated with 0.02 mL of a 21-25 day old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product from a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 2 minutes at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. The carriers were transferred to individual tubes containing 20 mL of DE Neutralizing Broth. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 60 days at 37±2°C. The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, confirmation of the challenge microorganism, sterility, viability, and neutralizer effectiveness.

Note: Protocol deviations/amendments reported in the study were reviewed.

6. MRID 484820-06 "AOAC Tuberculocidal Activity of Disinfectants Against *Mycobacterium bovis*, BCG" for Opti-Cide 3, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – November 2, 2009. Laboratory Project Identification Number A07968.

This confirmatory study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation, Durham, NC). One lot (Lot No. 31129N) of the product, Opti-Cide 3, was tested using ATS Laboratory Protocol No. MSI01060809.TB (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) porcelain penicylinder carriers for the single product lot were immersed for 15 minutes in a 21-25 day old suspension of the test organism, at a ratio of 1 carrier per 1.0 mL culture. The carriers were dried for 30 minutes at 35-37°C at 40% humidity. Each carrier was placed in 10.0 mL of the product for 2 minutes at 20.0°C. Following exposure,

the carriers were transferred to individual tubes containing 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 30 and 61 days at 35-37°C under aerobic conditions. The tubes were incubated for an additional 30 days because no growth was observed after 61 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

7. MRID 484820-07 "AOAC Use Dilution Test Against Vancomycin-resistant *Enterococcus faecalis* (ATCC 51299)" for Opti-Cide 3, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – January 21, 2011. Laboratory Project Identification Number 485-150.

This study was conducted against Vancomycin-resistant *Enterococcus faecalis* (ATCC 51299). One lot (Lot No. 33500) of the product, Opti-Cide 3 Disinfectant Cleaner, was tested using MicroBiotest Protocol No. 485.2.01.06.11 (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was placed in 10 mL of the product for 2 minutes at 20°C.The tubes containing the product were swirled after addition of the carriers. Following exposure, individual carriers were transferred to Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, confirmation of the challenge microorganism, sterility, viability, neutralizer effectiveness, bacteriostasis, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin-resistant *Enterococcus faecalis* (ATCC 51299) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 14 mm) confirmed antibiotic resistance of Vancomycin-resistant *Enterococcus faecalis* (ATCC 51299) to vancomycin. See pages 8 and 15 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

8. MRID 484820-08 "AOAC Use Dilution Test Against Methicillin-resistant *Staphylococcus aureus* (ATCC 33591)" for Opti-Cide 3, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – January 21, 2011. Laboratory Project Identification Number 485-149.

This study was conducted against Methicillin-resistant *Staphylococcus aureus* (ATCC 33591). One lot (Lot No. 33500) of the product, Opti-Cide 3 Disinfectant Cleaner, was tested using MicroBiotest Protocol No. 485.1.01.06.11 (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were

dried for 20-40 minutes at 37±2°C. Each carrier was placed in 10 mL of the product for 2 minutes at 20°C. The tubes containing the product were swirled after addition of the carriers. Following exposure, individual carriers were transferred to Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, confirmation of the challenge microorganism, sterility, viability, neutralizer effectiveness, bacteriostasis, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin-resistant *Staphylococcus aureus* (ATCC 33591) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin-resistant *Staphylococcus aureus* (ATCC 33591) to oxacillin. See pages 8 and 15 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed.

9. MRID 484820-09 "AOAC Use-Dilution Fungicidal Test Against *Trichophyton mentagrophytes* (ATCC 9533)" for Opti-Cide 3, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – February 14, 2011. Laboratory Project Identification Number 485-151.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). One lot (Lot No. 33500) of the product, Opti-Cide 3 Disinfectant Cleaner, was tested using MicroBiotest Protocol No. 485.3.01.06.11 (copy provided). The product was received ready-to-use. The product was not tested in the presence of a 5% organic soil load. Ten (10) stainless steel penicylinder carriers for the single product lot were immersed for 15 minutes in a 10-15 day old suspension of test organism, at a ratio of 20 carriers per 20 mL suspension. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was placed in 10 mL of the product for 2 minutes at 21°C. The tubes containing the product were swirled after addition of the carriers. Following exposure, individual carriers were transferred to Neopeptone Glucose Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. Tubes containing the neutralizer were shaken thoroughly after addition of the carriers. All subcultures were incubated for up to 10 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, carrier counts, confirmation of the challenge microorganism, sterility, viability, neutralizer effectiveness, and fungistasis.

10. MRID 484820-10 "Confirmatory Virucidal Efficacy Test Against Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)" for Opti-Cide 3, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – February 16, 2011. Laboratory Project Identification Number 485-152.

This confirmatory study, under the direction of Study Director S. Steve Zhou, was conducted against Duck hepatitis B virus (Strain LeGarth; obtained from HepadnaVirus Testing, Inc.), using primary duck hepatocytes (obtained from Metzer Farms) as the host system. One lot (Lot No. 33500) of the product, Opti-Cide 3 Disinfectant Cleaner, was tested according to a MICROBIOTEST protocol titled "Confirmatory Virucidal Efficacy Test - Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)," dated January 7, 2011 (copy provided). The product was received ready-to-use. The stock virus culture contained 100% duck serum. Films of virus

were prepared by spreading 0.4 mL of virus inoculum over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were exposed to 2.0 mL of the product for 2 minutes at 20°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, and 1% Hepes. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephacryl columns, and diluted serially in L-15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with the dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Following adsorption, the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

11. MRID 484820-11 "Confirmatory Virucidal Efficacy Test Against Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)" for Opti-Cide 3, by S. Steve Zhou. Study conducted at MICROBIOTEST. Study completion date – January 25, 2011. Laboratory Project Identification Number 485-153.

This confirmatory study, under the direction of Study Director S. Steve Zhou, was conducted against Bovine viral diarrhea virus (strain not specified; obtained from American Bioresearch Laboratories), using MDBK cells (ATCC CCL-22) as the host system. One lot (Lot No. 33500) of the product, Opti-Cide 3 Disinfectant Cleaner, was tested according to a MICROBIOTEST protocol titled "Confirmatory Virucidal Efficacy Test - Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)," dated January 7, 2011 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were exposed to 2.0 mL of the product for 2 minutes at 20°C. Following exposure, the plates were neutralized with 2.0 mL of horse serum with 1% Polysorbate 80 and 0.5% Lecithin. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephacryl columns, and diluted serially in Minimal Essential Medium with 5% horse serum. MDBK cells in multi-well culture dishes were inoculated eight-fold with the dilutions. The cultures were incubated for 5-7 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ carrier)
		Lot No. 21090	Lot No. 22010	Lot No. 22659N	
2-Minute Exposure Time					
484820-03	*Staphylococcus aureus*	0/60	0/60	0/60	2.9×10^6
	Salmonella enterica	0/60	0/60	0/60	3.5×10^6
	Pseudomonas aeruginosa	0/60	0/60	0/60	3.2×10^6
		Lot No. 33500			
484820-07	Vancomycin-resistant *Enterococcus faecalis*	0/10	---	---	3.2×10^6
484820-08	Methicillin-resistant *Staphylococcus aureus*	0/10	---	---	2.1×10^6
484820-09	*Trichophyton mentagrophytes*	0/10	---	---	2.6×10^5

MRID Number	Organism	Results		Plate Recovery Control
			Lot No. 33500	
2-Minute Exposure Time				
484820-10	Duck hepatitis B virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	$10^{4.50}$ and $10^{4.75}$ $TCID_{50}$/mL
		$TCID_{50}$/mL	$\leq 10^{1.50}$	
484820-11	Bovine viral diarrhea virus	10^{-2} to 10^{-3} dilution	Cytotoxicity	$10^{7.80}$ and $10^{7.93}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{4.80}$	
		Log reduction	≥ 3.0 $\log_{10}$	

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested	
			Lot No. 21330 90 Days	Lot No. 22010 90 Days
2-Minute Exposure Time				
484820-04	*Mycobacterium bovis* BCG Carrier Population: 2.6×10^5 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10
484820-05	*Mycobacterium bovis* BCG Carrier Population: 3.1×10^5 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10
			Lot No. 31129N 90 Days	
	Mycobacterium bovis BCG	Modified Proskauer-Beck Medium	0/10	---

MRID	Organism	Media	No. Exhibiting Growth/ Total No. Tested	
484820-06	Carrier Population: 6.3×10^5 CFU/carrier	Middlebrook 7H9 Broth†	0/10	---
		Kirchner's Medium†	0/10	---

†Middlebrook 7H9 Broth and Kirchner's Medium results could not be used to support product efficacy because neutralization confirmation testing failed to show growth of *Mycobacterium bovis* BCG for the single product lot in these two media.

MRID Number	Organism	Lot No.	Total No. Surviving (CFU/carrier)	Parallel Control (CFU/carrier)	Percent Reduction
10-Second Exposure Time; Hard, Non-Porous Surfaces					
484820-01	*Staphylococcus aureus*	21090	$<2.00 \times 10^1$	1.05×10^6	>99.9
		21330	$<2.00 \times 10^1$	1.05×10^6	>99.9
		30200	$<2.63 \times 10^1$	1.05×10^6	>99.9
	Enterobacter aerogenes	21090	$<2.63 \times 10^1$	2.24×10^6	>99.9
		21330	$<2.00 \times 10^1$	2.24×10^6	>99.9
		30200	$<2.63 \times 10^1$	2.24×10^6	>99.9
10-Second Exposure Time; Soft Surfaces					
484820-02	*Staphylococcus aureus*	21330	<1	1.58×10^5	>99.9
		31620	<1	1.58×10^5	>99.9
	Enterobacter aerogenes	21330	<1	1.38×10^5	>99.9
		31620	<1	1.38×10^5	>99.9

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, Opti-Cide 3 Disinfectant, as a disinfectant with bactericidal activity against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time:

Staphylococcus aureus	MRID 484820-03
Salmonella enterica	MRID 484820-03
Pseudomonas aeruginosa	MRID 484820-03

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted confirmatory efficacy data **support** the use of the product, Opti-Cide 3 Disinfectant Cleaner, as a disinfectant with bactericidal activity against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time:

Vancomycin-resistant *Enterococcus faecalis*	MRID 484820-07
Methicillin-resistant *Staphylococcus aureus*	MRID 484820-08

Complete killing was observed in the subcultures of the required number of carriers tested against the single product lot. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. The confirmatory studies tested one product lot, not the standard two product lots. This is acceptable and in accordance with Agency guidance for modifying label conditions (e.g., different exposure period).

3. The submitted confirmatory efficacy data (MRID 484820-09) **support** the use of the product, Opti-Cide 3 Disinfectant Cleaner, as a disinfectant with fungicidal activity against *Trichophyton mentagrophytes* on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time. Complete killing was observed in the subcultures of the required number of carriers tested against the single product lot. Neutralizer effectiveness testing showed positive growth of the microorganism. Viability controls were positive for growth. Sterility controls did not show growth. The confirmatory study tested one product lot, not the standard two product lots. This is acceptable and in accordance with Agency guidance for modifying label conditions (e.g., different exposure period).

4. The submitted confirmatory efficacy data **support** the use of the product, Opti-Cide 3 Disinfectant Cleaner, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of at least a 5% organic soil load (a 100% organic soil load against Duck hepatitis B virus) for a 2-minute contact time:

Duck hepatitis B virus	MRID 484820-10
Bovine viral diarrhea virus	MRID 484820-11

Recoverable virus titers of at least 10^4 were achieved. In studies against Bovine viral diarrhea virus, cytotoxicity was observed in the 10^{-2} and 10^{-3} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against Duck hepatitis B virus, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested. The confirmatory studies tested one product lot, not the standard two product lots. This is acceptable and in accordance with Agency guidance for modifying label conditions (e.g., different exposure period).

5. The submitted efficacy data (MRID 484820-04 and 484820-05) **support** the use of the product, Opti-Cide 3 Disinfectant, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time. Complete killing was observed in the subcultures of the required number of carriers against the required number of product lots. No growth was observed in the subcultures of the two extra media. Neutralizer effectiveness testing showed positive growth of the microorganism. Viability controls were positive for growth. Sterility controls did not show growth.

6. The submitted confirmatory efficacy data (MRID 484820-06) **support** the use of the product, Opti-Cide 3, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time. Complete killing was observed in the subcultures of the required number of carriers against a single product lot. No growth was observed in the subcultures of the two extra media. Neutralization confirmation testing showed positive growth of the microorganism in Modified Proskauer-Beck Medium. [Neutralization confirmation testing showed no growth of the microorganism in Middlebrook 7H9 Broth and Kirchner's Medium.] Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

7. The submitted efficacy data **support** the use of the product, Opti-Cide 3, as a sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 10-second contact time:

Staphylococcus aureus	MRID 484820-01
Enterobacter aerogenes	MRID 484820-01

Bacterial reductions of at least 99.9 percent over the parallel control were observed within 5 minutes (i.e., 10 seconds specifically). At least one of the product lots tested was at least 60 days old at the time of testing. The parallel control demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Sterility controls did not show growth.

8. The submitted efficacy data **support** the use of the product, Opti-Cide 3, as a sanitizer against the following microorganisms on pre-cleaned, soft non-food contact surfaces for a 10-second contact time:

Staphylococcus aureus	MRID 484820-02
Enterobacter aerogenes	MRID 484820-02

Bacterial reductions of at least 99.9 percent over the parallel control were observed within 5 minutes (i.e., 10 seconds specifically). At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Sterility controls did not show growth.

VII. LABEL

1. The proposed label claims that the product, Opti-Cide 3, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time:

Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus
Vancomycin-Resistant *Enterococcus faecalis*
Methicillin-Drug Resistant *Staphylococcus aureus*

Trichophyton mentagrophytes

Duck hepatitis B virus
Hepatitis B virus
Bovine viral diarrhea virus
Hepatitis C virus

Mycobacterium bovis BCG

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Opti-Cide 3, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a **2-minute contact time**:

Herpes simplex II virus
Human immunodeficiency virus
Influenza virus, Strain A2/Hong Kong

Data were not provided to support these claims. The label must be revised to list these microorganisms as requiring a 3-minute contact time.

3. The proposed label claims that the product, Opti-Cide 3, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 10-second contact time:

Staphylococcus aureus
Enterobacter aerogenes

These claims are acceptable as they are supported by the submitted data.

4. The proposed label claims that the product, Opti-Cide 3, is an effective sanitizer against the following microorganisms on pre-cleaned, soft non-food contact surfaces for a 10-second contact time:

Staphylococcus aureus
Enterobacter aerogenes

These claims are acceptable as they are supported by the submitted data.

5. The following revision must be made to the proposed label:

- Under the "Directions for Use" section of the proposed label, identify how the product is to be applied (e.g., by sponge, mop, spray, immersion), followed by a statement such as "to wet all surfaces thoroughly."

6. The Data Matrix does not identify efficacy studies for the following: Canine Distemper virus (ATCC VR-128), Canine parainfluenza virus (ATCC VR-666), Feline leukemia virus, *Streptococcus equi*, and Canine parvovirus.